

# Properties of ribosomes

0 universal occurrence : amount correlated with protein synthesis.  
 1 ~~are~~ mainly RNA + protein ~ 60 : 40 in coli  
 50 : 50 in higher organism.

2 contain most of the RNA of a cell.

3 discrete sizes. Scheme for coli depends upon  $Mg^{++}$  conc.  $\leftarrow ?$

	30S	50S	$\Rightarrow$	70S	<del>100S</del>	$10^6$	30	50	70S	?
							1.0	1.8	3.1	

270S  $\rightleftharpoons$  600S.

4 size of RNA



30



50



~ 16S  $0.6 \times 10^6$   
 ~ 23S  $1.2 \times 10^6$

composition of RNA (high G, med. A, C, low U.)  
 50% at 30S

RNA shows some methylation.

5 protein composition. Same. *coa* (histone)  
 comp. similar in all species.  
 subunits. *adp* (25,000)  
*Mer, Ale + ...*

6 Shape of ribosome.

20S



Similar in other species?  $\leftarrow ?$

probably heavily hydrated.

lactar RNase on 30S particle (degrades to 2' 3')  
 cleaves to cytosine  $\rightarrow$  2' 3'  
 then to more slowly to 3' (some shift in rates for diff bases)  
 amount? ~~part~~ deals with ribosome breakdown?  
 other lactar enzymes?

are all ribosomes the same? theory of ribosome structure.

Nattam and Lipmann (PNAS) 1961 47 497

Amino acid transfer from aminoacyl-RNAs to protein on ribosomes of E. coli

E. coli used ~~2~~ <sup>loaded with Leu\*</sup> S-RNA to show transfer

to show requirement for a factor ribosomes must be "washed"  
(Spin 150,000 g for <sup>3</sup> hours)

One wash : shows some requirement

3 washes : show - abs. ....

Factor is non-dialyzable : heat labile (1 min at 70°C)  
rather unstable.

purified : some av. enzymes like, but no Leu activity activity.

appeared to be one factor for "all" a.a.

since peak comes off DEAE-cellulose was in same place  
for transfer of Leu, Lys, Phe, Val and Tyr. (other claim for  
not true by others)

Species specificity: protein made with rat liver factor had shown  
no specificity ~~between~~ { rabbit, pigeon, chicks or calf liver  
or rabbit retics. all could replace  
rat supernatant.

but rat and E. coli wouldn't work (though E. coli S-RNA used).  
(Siml. result by Rendli + Ochoa)

Condition for transfer : high (0.14M) Mg<sup>++</sup> optimal.

GSH helps somewhat.

GTP needed. PEP + kinase needed.

inhibited by puromycin + chloramphenicol.

not re-act by old Leu. (and free Leu\* not incorp,  
even if ATP included)

P.S. knew that GTP

split in 2 aa incorp.

two factors

~~recovered~~ recovered S-RNA appears unaltered

an enzymatic deacylation reaction, needs ribosomes, and <sup>occurs</sup> : with puromycin this is  
increased.  
(also some loss by stray hydrolysis)

occurrence of protein synthesis: how far is general scheme applicable?

nucleus: system similar to cytoplasmic, except  $\text{Mg}$  dependent system to amino acid transfer, and DNA (or other polyanion) necessary for ATP supply.

believed to contain "ribosomes", and activating enzymes and S-RNA (may be different)

Cytoplasm endoplasmic reticulum, in some cells. mainly in cells which secrete. (claim that ribosomes plus lipid do better). "microsomes" use of deoxycholate to remove lipid, etc. but ribosomes universal.

role of end. retic not clear, but suggests it may help protein release. (serum albumin in rat liver) can be released (after *in vivo* work by Doc, or alternative, from microsomes, but not from ribosomes)

Mitochondria: definitely  $\text{aa}^+$  uptake into protein, but not into cytochrome C or catalase. (RNAse *in situ* - needs energy supply)

mit. contain some 1% RNA. if incorp done on intact mit. + then broken open, most  $\text{aa}^+$  counts in RNA-rich 100,000s sediment, but no ribosomes seen so far. acetone powder of mit. appear to contain acc. enz. (PP<sup>+</sup> kinase) aa dep.

Chloroplasts: some  $\text{aa}^+$  incorp. RNA? prob. ribosomes??

General scheme. (having described propn. of major component)

Act. enzyme. } ATP + ~~general~~ (T-RNA acts  
S-RNA or T-RNA } catalytically)  
~~GTP~~ GTP + transfer factor(s) + ATP generating system.

ribosome + messenger RNA - LATER.

release process? ATP dependent?

possible to see, in a cell-free system, synthesis (or finishing) of a <sup>well-</sup>detected protein.

remarks: dwell on specificity rather than on the biochemical steps.

"Special" systems <sup>early days.</sup> rat liver. (serum albumin)  
→ (see seedling)

- E. coli.

- reticulocytes HB.